Nitrogen metabolism of beef steers fed endophyte-free tall fescue hay: Effects of ruminally protected methionine supplementation[1]

S. L. Archibeque*†, J. C. Burns*‡§, and G. B. Huntington*†[2]

*Animal Science Department, †Interdepartmental Nutrition Program, ‡USDA-ARS, and §Crop Science Department, North Carolina State University, Raleigh, NC 27695

ABSTRACT: Level of nitrogen (N) intake and ruminally protected methionine supplementation were evaluated in eight Angus growing steers (initial BW 253 ± 21 kg, final BW 296 ± 21 kg) in a replicated 4 ± 4 Latin square design. The steers were fed two endophyte-free tall fescue (*Festuca arundinacea*) hays that contained 2.2 (LO) or 2.8 % (HI) of DM as N and were either supplemented or not with ruminally protected methionine (10 g metabolizable methionine/d). Diets were fed to provide adequate energy for 0.5 kg ADG and sufficient protein for maintenance (LO), or protein to support 0.5 kg ADG (HI). Following at least 14 d of adjustment, N balance was measured for 6 d. Isotopic urea was infused ($^{15}N^{15}N$-urea, 0.164 mmol urea N/h) via a jugular catheter for 56 h and urine was collected from 48 to 56 h to measure urea kinetics. Jugular blood was collected during the balance trial, and serum was analyzed for serum urea N (SUN). By design, daily N intake was greater ($P < 0.05$) for HI (112 g) than for LO (89 g). Compared with LO, steers when fed HI had greater ($P < 0.05$) daily DMI (4,217 vs 4,151 g), fecal N (34.4 vs 31.2 g), N digested (77.1 vs 57.7 g), urine N (48.3 vs 37.5 g), urine urea N excretion (34.6 vs 24.8 g), and N retained (29.8 vs 21.1 g). When fed HI steers also had higher ($P < 0.05$) urine urea N concentration (276 vs 219 m*M*), SUN (8.7 vs 6.7 m*M*), N digestibility (69.1 vs 64.9 %), percentage of urinary N present as urea (71.5 vs 66.7%, $P < 0.053$), and rate of urea N production (59.6 vs 49.2 g/d) but lower ($P < 0.05$) percentage of urea N produced that was returned to the ornithine cycle (15.03 vs 19.2 1%) than when fed LO. Methionine supplementation decreased daily urine N (41.2 vs 44.6 g, $P = 0.10$) and increased both the amount of N retained daily (27.9 vs 23.7 g, $P < 0.089$) and the percentage of N digested that was retained (40.4 vs 34.6 %, $P < 0.094$). In summary, supplemental methionine met a specific dietary limitation by increasing the amount of digested N that was retained by the steers.

Key Words: Beef Cattle, Nitrogen Metabolism, Methionine, Festuca

 J. Anim. Sci. 2002. 80:1344–1351

Introduction

Archibeque et al. (2001) studied the effects of N intake in grass hay diets on N metabolism of steers. Although N digestibility increased as N intake increased, there was no difference in N balance between the two levels of N intake. These data indicate that some limitation in postabsorptive metabolism may have decreased the efficiency of N retention of the steers. This could be due to an amino acid deficiency or imbalance at the tissue level. Because methionine is typically considered to be the first-limiting amino acid for beef cattle (Merchen and Titgemeyer, 1992; Klemesrud et al., 2000), along with other sulfur amino acids, it is a valid choice for a supplement to alleviate an amino acid deficiency. A ruminally protected methionine supplement increases the proportion of dietary amino acid that is absorbed from the intestine. This is accomplished by encapsulating the methionine in a pH-sensitive coating that is stable in the rumen but unstable when it enters the abomasum, thereby making methionine available for intestinal digestion and absorption (Polan et al., 1991). If the absorbed methionine meets a critical limitation and improves the overall use of N in the diet that is converted to a saleable product such as muscle, leather, or wool, with less N being excreted, then there is more potential to produce a profit while minimizing undesirable environmental impacts through modifications in urea kinetics. The objective of this study was to decrease urea production and excretion and increase the amount and efficiency of N retention of growing beef steers by supplementing their diet of endophyte-free tall fescue hay with ruminally protected methionine.

[1]This research was supported in part by the North Carolina Inst. of Nutrition.

[2]Correspondence: phone: 919-513-1693; fax: 919-515-4463; E-mail: gerald_huntington@ncsu.edu.

Received June 20, 2001.

Accepted January 8, 2002.

Table 1. Ingredient composition of supplement with no (NM) or with (MS) ruminally protected methionine

	% DM	
Ingredient	NM	MS
Cracked corn	90.15	84.16
Mono-dicalcium phosphate	5	5
$CaCO_3$	2.82	2.82
Trace mineral salt[a]	1.68	1.68
Vitamin premix[b]	0.34	0.34
$CuSO_4$	0.02	0.02
Met-Plus[c]	0	5.979

[a]Mg (5680 mg/kg) as MgO; Zn (170 mg/kg) as $ZnSO_4$; Co (0.57 mg/kg) as $CoCO_3$; Se (0.57 mg/kg) as Na_2SeO_4.
[b]Contained per kilogram supplement DM: 5,000 IU of Vitamin A, 625 IU of Vitamin D, and 114 IU of Vitamin E.
[c]Ruminally protected methionine source, 65% DL-methionine.

Materials and Methods

Animals and Experimental Procedures

A 4 ± 4 Latin square design was used with a 2 ± 2 factorial arrangement of two endophyte-free tall fescue (*Festuca arundinacea*) hays with (**MS**) or with no (**NM**) ruminally protected methionine supplementation (Met-Plus, Nisso-America, New York). Hays were harvested at the late vegetative stage of growth from the same field on the same day. One hay with high N (**HI**) was dried with heated, forced air as described by Burns et al. (1997), and the second hay with low N (**LO**) was dried with solar radiation.

Eight Angus growing steers (initial BW 217 ± 15 kg, final BW 252 ± 9 kg) were used in this experiment. The North Carolina State University Animal Care and Use Committee approved care, handling, and sampling of these steers. Steers were obtained from the university herd, trained to be led by halter, and accustomed to close human interaction. Following 14 d of adaptation to their indoor facilities and exercise lot, steers were blocked by weight into two groups and transported to an indoor facility where they were housed in individual tie stalls (115 ± 178 cm). The steers were allowed to exercise in an outdoor pen on a regular basis (two to three times a week for 3 to 4 h) between collection periods. Steers were adapted to the facilities with a diurnal pattern of 12 h of light and 12 h of dark for at least 14 d, during which time they received the experimental hays in a randomized fashion, with no steer receiving a given hay 2 d in a row. Steers received a supplement (NM, Table 1) formulated to provide all necessary vitamins and minerals for desired growth (0.5 kg/d, NRC, 1996) and had ad libitum access to water.

Diets were formulated to provide adequate ME for 0.5 kg ADG. Metabolizable protein supply was near requirements for maintenance (LO) or for 0.5 kg ADG (HI) for a 250-kg growing steer (NRC, 1996). As such, 3.78 kg DM hay and 0.44 kg DM supplement were offered daily. The hay was offered in two equal portions at 1000 and 1630, and the supplement was offered with the 1000 feeding. Following adaptation to facilities, steers were randomly assigned to a rotation of the four treatments. Methionine supplementation consisted of 25 g/d of Met-Plus in the corn supplement to provide approximately 9.9 g/d of metabolizable methionine. This is based on the assumptions that 65% of the supplement is D,L methionine, 67.4% of the methionine is not degraded in the rumen, and that methionine has an intestinal absorption coefficient of 90.4% (Bach and Stern, 2000). Steers were adapted to each experimental diet for at least 14 d. Prior to collections, all pens to be used for collections were thoroughly scrubbed and washed. Separation boards, designed to allow visual contact among steers, were attached to pens to minimize cross-contamination of feces between steers. Steers were then fitted with two temporary catheters in the same exterior jugular with tips 10 cm apart in the vena cava. The catheter closest to the heart was used for infusions and the other catheter was used for blood sampling. Integrity of catheters was maintained with Na-heparin (100 units/mL 0.9% sterile saline). Total collections of urine, feces, and orts were conducted for 6 d following adaptation to the respective diet as described by Archibeque et al. (2001).

Following 24 h of total collection, blood and urine samples were collected to establish baseline enrichments of ^{15}N. Urea kinetics were measured during d 2 to 4 of the balance trial. Steers were then infused via the caudal jugular catheter with $^{15}N^{15}N$-urea (Cambridge Isotope Laboratories, Andover, MA; Lot T1-4560) prepared in sterile 0.9% NaCl saline. The infusion rate was maintained at 85 mL/h, which delivered 0.164 mmol of urea N/h using a peristaltic pump (Model 1000, Medical Technology Products, Huntington Station, NY). Urine was collected at 2-h intervals in a separate vessel from 48 to 56 h of infusion for determination of ^{15}N enrichments, and the remaining urine was transferred to the vessel for total collection. Samples taken for ^{15}N analysis were accounted for in the total collection data. Plateau enrichments were confirmed by analyzing the enrichment data using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) to regress the data over the time-course of sample collection. This plateau was achieved ($P > 0.10$) at 0.13 atoms percent excess (SE = 0.005).

Chemical Analysis

All feed, orts, and feces samples were ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass through a 1-mm screen and stored at room temperature in sealed containers until analyzed. Urine samples were stored in 30-mL plastic bottles and frozen at < –4°C until analyzed. Duplicate samples of feed, orts, and feces were analyzed for DM, ash, and Kjeldahl N using AOAC (1984, 1999) procedures. Neutral detergent fiber, ADF, and 72% sulfuric acid residue of forage samples were sequentially determined using the method of Van

Soest et al. (1991) without added amylase or urea in a batch processor (Ankom Technology Corp., Fairport, NY). In vitro true dry matter digestibility (**IVTDMD**) was determined by an in vitro incubation of 0.25-g samples in Ankom fiber bags (Ankom Technologies, Fairport, NY) for 48 h. Samples were inoculated with 1,600 mL of McDougal's buffer (Tilley and Terry, 1963) and 400 mL of strained ruminal fluid, from a fistulated steer on a alfalfa hay ration, using the Ankom II Daisy batch fermenter (Ankom Technologies). In vitro fermentations were terminated with the NDF procedure in the Ankom 200 fiber analyzer to remove the residual microbial fraction. Urea content of serum, urine, and $^{15}N^{15}N$-urea infusate were analyzed using the diacetyl monoxime method of Marsh et al. (1957) using a Technicon Auto Analyzer (Technicon Instruments, Tarrytown, NY). Protein fractions of the forages were determined as described by Licitra et al. (1996), whereby the N contained in the forage is classified by the type of fiber with which it is associated. Nonprotein N, or the A fraction of CP, was determined as the difference between total Kjeldahl N and the Kjeldahl N of the residue remaining after using a 10 % (wt/vol) trichloroacetic acid precipitation. The subsequent B_1, B_2, B_3, and C fractions were determined by refluxing in acid or neutral detergent followed by N determination of the residue. Protein fractions were determined on composite samples of forages and analyzed in triplicate.

Calculations of Urea Kinetics

Analysis of ^{15}N urea enrichment of urine was conducted as described by Archibeque et al. (2001). The enrichments of urea with a mass-to-charge ratio of m/z 29 and m/z 30 were then applied to the model of Sarraseca et al. (1998) to determine urea kinetics. The basic concept is that the infused $^{15}N^{15}N$-urea is diluted into the circulating blood until a plateau of enrichment is achieved. This dilution is used to determine the total urea synthesis, or urea-N entry rate (**UER**). When $^{15}N^{15}N$-urea enters the rumen, it is hydrolyzed by microbial urease, yielding two $^{15}NH_3$ molecules. The rate at which urea enters the GIT (**GER**) is calculated to be the difference between UER and urea excretion in the urine. The $^{15}NH_3$ molecules produced by urease can then either be used by bacteria for protein synthesis or are absorbed back into circulation. If these molecules are reabsorbed as such, they are transported back to the liver, where they are combined with ^{14}N atoms (aspartate) in the hepatic ornithine cycle, yielding $^{14}N^{15}N$-urea, in proportions based on the laws of probability relating to natural ^{15}N abundance (Jackson et al., 1984; Sarraseca et al., 1998). The rate at which this NH_3 N is returned to the ornithine cycle is labeled **ROC.** Based on these assumptions, the resulting enrichments of m/z 29 and m/z 30 derived from urine urea were applied to the model developed by Sarraseca et al. (1998), which accounts for multiple re-entries of urea through the system.

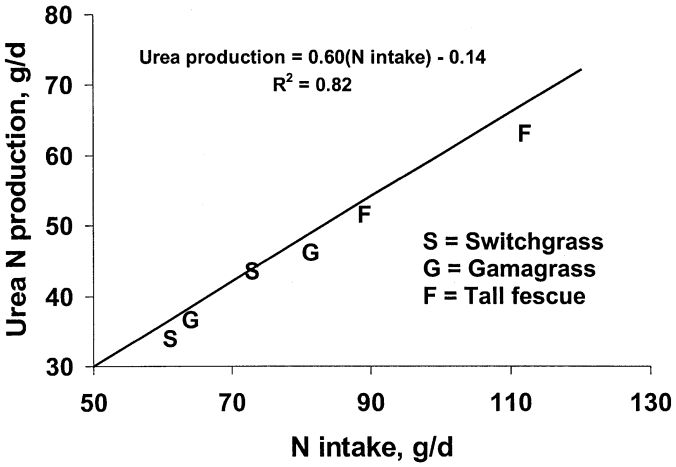

Figure 1. Urea production rate as a function of N intake in steers fed different forages.

There was insufficient HI hay to complete both replicates of the Latin square design. As a result, six steers received all diets, and two steers from one square received all diets except for one HI treatment. One steer disassembled its catheters within 1 h before the first samples for the urea kinetics were taken, while receiving the HI, MS treatment. The kinetic data from that steer were removed from the data set, but the N balance data from this steer were included in the data set. Therefore, n = 6 for all dependent variables for the HI-NM treatment, n = 5 for the kinetic data for HI-MS, and n = 8 for all dependent variables for the other two treatments.

Statistical Analysis

Statistical analyses of data were performed using analyses of variance for a Latin square design and the GLM procedure of SAS. The model for balance trial data and urea kinetic data included the independent variables of square, animal(square), period(square),

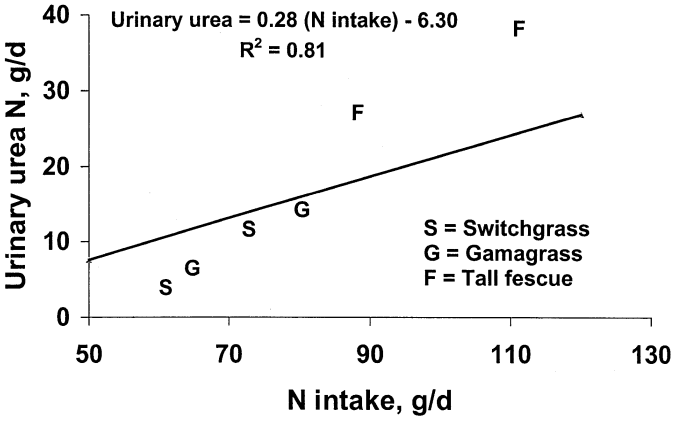

Figure 2. Urinary urea production as a function of N intake in steers fed different forages.

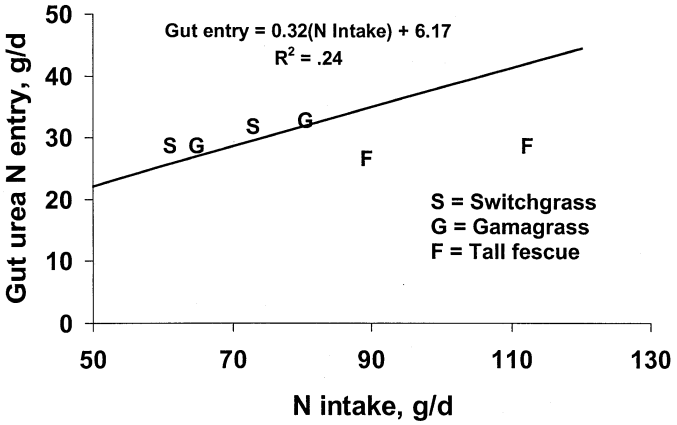

Figure 3. Gut urea N entry rate as a function of N intake in steers fed different forages.

forage, supplementary methionine level, and forage × supplementary methionine level interaction. Treatments were considered to differ when $P \leq 0.10$.

Several linear relationships among diet composition and urea metabolism were derived from combining present data with those from a similar study with warm-season grasses (Figures 1–6). The data set included eight observations from the present experiment (means of steers for LO and HI across methionine supplementation) and 16 observations from steers fed two levels of N intake in gamagrass and switchgrass hays (Archibeque et al., 2001). Statistical analyses of these data were done with the GLM procedure of SAS. The analysis contained forage as a class variable, N intake (or N fraction intake) as a covariate, and the forage × N intake interaction (St-Pierre, 2001).

Results

Steers when fed HI received 7.62 g/d more NPN (A fraction) and 14.8 g/d readily degradable N (B_2 fraction) than when fed LO (Table 2). This was accompanied by a decrease of 2.4 g/d of slowly degradable, insoluble N (B_3 fraction) and an increase in IVTDMD (Table 2) in steers when fed HI than when fed LO.

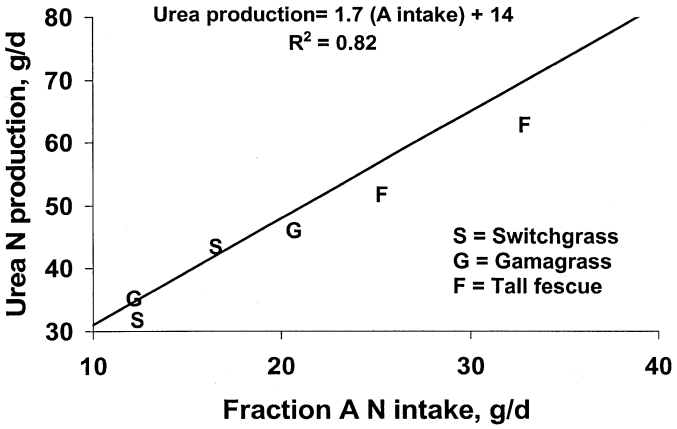

Figure 4. Urea production rate as a function of fraction A N intake in steers fed different forages.

Table 2. Fiber characterization, in vitro true dry matter digestibility, and protein fractionation of tall fescue hay with high (HI) or low (LO) N concentration

Item, % of DM	HI	LO	SE	Difference in N intake g/d[d] (HI – LO)
Hemicellulose	29.2	30.0	0.35	—
Cellulose	26.3	28.7	0.20	—
Lignin	2.55	2.7	0.10	—
IVTDMD	79.7	75.7	0.62	—
CP	17.5	14.3	0.27	—
N Fractions[bc], % of CP				
A	30.7	29.9	7.62	
B_1	2.95	1.25	2.07	
B_2	43.9	38.0	14.8	
B_3	20.7	29.3	–2.40	
C	1.82	1.63	0.58	

[a]Least squares means from a complete randomized design.
[b]Using the method of Licitra et al. (1996).
[c]Arithmetic means of triplicate composite samples.
[d]Calculated as (N intake from HI hay, g/d) × N fraction – (N intake from hay, g/d) × N fraction.

No interactions were detected in N intake, digestion, retention variables, SUN (Table 3), or in urea kinetic parameter estimates (Table 4). In response to increased N intake (HI vs LO), DMI increased 66 g/d ($P < 0.01$), N intake increased 22.7 g/d ($P < 0.01$), fecal N increased 3.26 g/d ($P < 0.01$), N digested increased 19.4 g/d ($P < 0.01$), urinary N increased 10.75 g/d ($P < 0.01$), and N retention increased 8.67 g/d ($P < 0.01$). Similarly, in response to increased N intake (HI vs LO), UER increased 11.1 g/d ($P < 0.01$), SUN increased 2 mM ($P < 0.01$), urine urea N increased 9.77 g/d ($P < 0.01$), urea as a percentage of total urinary N increased 4.82 percentage units ($P < 0.053$), and ROC as a percentage of UER decreased 4.1 percentage units ($P < 0.01$).

Methionine supplementation (MS vs NM) tended to decrease ($P = 0.10$) urinary N by 3.34 g/d, tended to increase ($P < 0.089$) N retained by 4.11 g/d, and tended to increase ($P < 0.094$) the proportion of the digested N that was retained by 5.73 percentage units (Table 3). Supplementation of ruminally protected methionine did not affect urea kinetic parameter estimates (Table 4).

Discussion

The hays used in this study were dried with different techniques to create two levels of N (HI and LO) without radically altering the amount of energy available to the steers. It appears that most of this N increase in HI vs LO was due to N present as insoluble, readily degradable N and as NPN, as well as a decrease in the amount of N present as slowly degradable, insoluble N (Table

Table 3. Intake, digestion, retention and plasma urea N in steers fed tall fescue hay dried with forced air (HI) or with solar radiation (LO)[a] with no (NM) or with (MS) ruminally protected methionine supplementation

	HI		LO			*P*-value		
Item	NM	MS	NM	MS	SE[b]	Forage	Methionine	F × M[c]
DMI, g/d	4,218	4,216	4,152	4,150	20	0.01	0.93	0.99
N Intake, g/d	111	112	88.3	89.5	1.2	0.01	0.39	0.83
Feces DM, g/d	1155	1200	1182	1166	23	0.89	0.56	0.23
Feces N, g/d	34.0	34.8	31.3	31.0	0.8	0.01	0.76	0.50
Urine N, g/d	50.3	46.3	39.0	36.2	2.1	0.01	0.10	0.76
Urine Urea N, g/d	35.8	33.4	26.0	23.6	1.7	0.01	0.16	0.97
Urea, % of urine N	70.7	72.3	67.1	66.2	2.4	0.05	0.86	0.57
DM digestibility, %	69.1	67.8	68.1	68.5	0.7	0.82	0.54	0.21
N digested, g/d	77.1	77.2	57.0	58.5	1.3	0.01	0.51	0.53
N digestibility %	69.4	68.9	64.4	65.3	0.8	0.01	0.84	0.34
N retained, g/d	27.8	31.8	19.0	23.3	2.4	0.01	0.09	0.95
N retained, % of N digested	36.3	41.1	33.0	39.6	3.5	0.47	0.10	0.78
Serum urea N, m*M*	8.8	8.6	6.8	6.7	0.2	0.01	0.39	0.81

[a]Least squares means from eight steers in a replicated, incomplete Latin square design.
[b]Largest standard error of unbalanced data is reported.
[c]Forage × methionine interaction.

2). Alterations in N fractions were associated with decreased structural carbohydrate in HI vs LO, which led to a higher IVTDMD, as would be expected. However, this effect was not apparent in the steers, which had a similar apparent DM digestibility between hays (Table 3). Apparently, the microbial contribution to fecal DM in the steers created enough variation to mask differences in digestibility due to differences in content of structural carbohydrates.

Effects of N Intake and Methionine Supplementation on N Digestibility and Whole-Body N Metabolism

By design, there was a greater daily intake of N in steers when fed HI than when fed LO, and for the same reason there was no difference in N intake between the NM and MS (Table 3). As a result, any differences seen with methionine supplementation were due to increased postruminal methionine supply and not due to increased total N supply. Our protein fraction analysis indicated that there was an increased amount of the B_2 fraction in the HI hay, a decrease in the B_3 fraction (Table 2), and an increase in the amount of fecal N produced (Table 3). Whereas there was an increase of 19.4 g/d in digested N in HI vs LO and fecal N increased 3.2 g/d, there was a decrease in the B_3 fraction and only 0.58 g/d more N in the C fraction in Hi vs LO. Apparently the increased amount of N in HI vs LO was not used as efficiently for anabolic processes (even though it should have been more available), or there

Table 4. Urea kinetic parameter estimates[ab] of steers fed tall fescue hay dried with forced air (HI) or with solar radiation (LO) with no (NM) or with (MS) ruminally protected methionine supplementation

	HI		LO			*P*-value		
Item	NM	MS	NM	MS	SE[c]	Forage	Methionine	F × M[d]
Urea transfers, g N/d								
Urea-N entry rate	63.9	61.6	51.6	51.8	2.96	0.01	0.68	0.63
GIT[e] entry	29.4	28.2	25.6	28.2	3.61	0.56	0.81	0.54
Recycled from GIT	10.2	9.0	10.2	9.7	1.16	0.72	0.40	0.72
Percentage contributions								
GIT entry: production	45.1	45.3	49.0	53.1	3.98	0.12	0.53	0.56
Recycling: GIT entry	35.4	33.5	41.4	35.5	4.33	0.31	0.31	0.60
Recycling: production	15.6	14.4	19.8	18.6	1.61	0.01	0.38	0.99

[a]As measured using a continuous infusion of bis^{15}N-urea.
[b]Least squares means from eight steers in a replicated, 4 × 4 Latin square design.
[c]Largest standard error of unbalanced data is reported.
[d]Forage × methionine interaction.
[e]GIT = gastrointestinal tract.

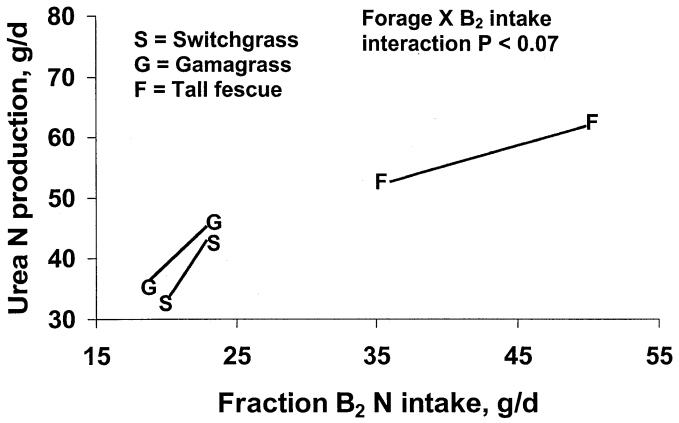

Figure 5. Urea production rate as a function of fraction B_2 N intake in steers fed different forages.

was a greater postruminal entry of urea N into the gastrointestinal tract. Postruminal transfer of urea N into the gastrointestinal tract can be substantial and correlates to plasma urea concentration and urea production rate (Norton et al., 1978; Kennedy, 1980), both of which were greater in steers when fed HI than when fed LO (Table 4).

The increase in N intake when steers were fed HI, particularly the A fraction, would lead to a greater production of ruminal ammonia, which is proportional to the amount of soluble N (Wohlt et al., 1976). This increase in the ruminal ammonia pool should lead to an increase in the amount of readily available N for proliferation of ruminal microbes; Hristov and Broderick (1994) calculated that 40 to 68% of microbial protein could come from ammonia. This would increase the amount of protein available to the postruminal gastrointestinal tract for digestion and absorption (Table 3). As a result, the steers retained more N when fed the HI vs LO.

Besides to the additional ammonia for microbial proliferation, increased N intake increased absorption of ammonia, or irreversible loss from the rumen, which in turn increased entry of ammonia into the circulation (irreversible loss of ammonia from the rumen, g/d = 3.829 + 0.507(intake, g/d), R^2 = 0.853, Parker et al., 1995). The blood was detoxified of this circulating ammonia, primarily by the hepatic ornithine cycle (Meijer et al., 1985; Huntington, 1989). This was accompanied by the increased concentration of SUN and, as a result, there was an increased amount of urea excreted in the urine (Table 3).

Reduced urinary N and increased N retained as a percentage of intake or as a percentage of N digested for MS vs NM (Table 3) support the concept that methionine met a specific methionine and(or) sulfur amino acid deficiency. This is in accord with the theory that methionine is typically considered to be the limiting amino acid for beef cattle (Merchen and Titgemeyer, 1992).

The similarity in fiber composition among the forages (Table 2) allowed for equal digestibility of the diets. Supplementation of the ruminally protected methionine did not alter apparent total DM or N digestion. However, there was an increase in the amount of N digested when steers were fed the HI level of N (Table 3). This was a result of more total N present to be digested as well as an increase in the amount of N present in the more digestible fractions (Table 2). The increased proportion of N that was present in the more digestible fractions of HI vs LO also led to increased apparent digestibility.

Effects of N Intake and Methionine Supplementation on Urea Metabolism and Recycling

Urea production increases with increased N intake (Bunting et al., 1987; Huntington, 1989), even when such increases are coupled with increases in energy intakes (Sarraseca et al., 1998). If the diet is inadequate to provide the necessary constituents for growth, the body will begin to degrade other tissues to meet specific needs. This has been established for both ruminants (Whitelaw et al., 1990) and nonruminants (Meakins and Jackson, 1996). Therefore, if diets are inadequate in nutrients or if dietary intake of N exceeds the metabolic capacity to retain N, then urea production will increase. Our steers demonstrated the expected increase in urea production with the increased intake of dietary N (Table 4). However, the lack of effect due to methionine supplementation on all the urea kinetic variables (Table 4) suggests that any N spared by meeting a methionine requirement did not change the rate at which N was cycled into the urea pool. This is in contrast to the results of Bach et al. (2000), who demonstrated a decrease in both urea production and entry into the gastrointestinal tract in dairy cows supplemented with the same commercial source of ruminally protected methionine. Compared to our steers, the dairy cows in that study received more than twice as much

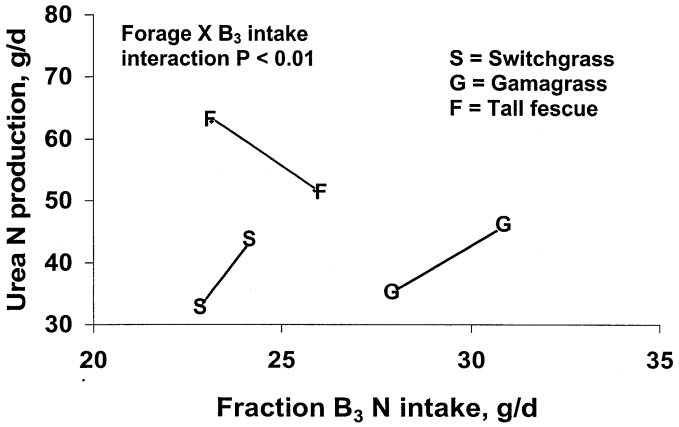

Figure 6. Urea production rate as a function of fraction B_3 N intake in steers fed different forages.

daily N intake and almost twice as much daily methionine, but less methionine as a percentage of body weight. Nonetheless, it may be that our level of methionine supplementation was inadequate to elicit a response or our system was not sensitive enough to detect it, or only offering the supplement once a day masked the effect due to a temporal methionine excess. The relatively higher N output of dairy cows vs weight-gain of beef steers may have allowed the response in the cows, whereas our steers' weight gain was limited by other factors, precluding the detection of this response.

The values for percentage of urea N that entered the gastrointestinal tract that was subsequently recycled to reform urea in this study (34 to 41%) were similar to those (36.9 to 40.7%) of Sarraseca et al. (1998), who fed yearling goats various intakes of a grass pellet diet. This suggests that between 59 and 66% of the urea N that entered the rumen was incorporated into microbial constituents for later use by the steers. However, these values are slightly greater than those obtained by Bunting et al. (1987), who reported that only 55 to 58% of the BUN that entered the rumen was incorporated into microbial N in lambs fed a corn-based diet. Conversely, a study of urea kinetics in steers fed warm-season grasses found 65 to 70% of BUN that entered the gastrointestinal tract could be incorporated into microbial N (Archibeque et al., 2001). Huntington et al. (1996) found that, as the amount of concentrate in the diet increased, the rate at which urea N entered the gastrointestinal tract decreased when steers were fed near or below maintenance N intakes. Therefore, in forage-fed animals, there is typically a greater rate of urea N entering the gastrointestinal tract, with a greater amount being absorbed postruminally (Huntington et al., 1996), allowing for a greater possibility for this N to be incorporated into microbial N throughout the entire gastrointestinal tract. Because there is a steady (or a less variable) proportion of ammonia N that is being reabsorbed and converted back to urea N (ROC), then the more notable variations in urea production between levels of dietary N intake will be responsible for this difference. Therefore, urea kinetics of these beef steers were altered primarily through changes in UER.

Summation of the Relationship Between N Intake and N Metabolism Among Experiments

The present experiment and that of Archibeque et al. (2001) provide data for comparing forages (fescue, gamagrass, and switchgrass, with adjustment for differences in N intake among the forages) and for evaluating potential interactions between forages and level of intake of N components.

Both urea production rate (Figure 1) and urinary urea excretion (Figure 2) exhibited strong, positive linear relationships with increasing N intake. The relationship between N intake and gut urea entry (Figure 3) had a lower correlation coefficient than that for urea production or urinary excretion, which may reflect differences among forages in ruminal ammonia concentrations that accompany increased N intakes (Rémond et al., 1993). After adjustment for differences in N intake, forages differed ($P < 0.01$) in urinary urea production, but not in urea production or gut urea entry. The slopes from these three regressions indicate that approximately 60% of each increment in dietary N is incorporated into urea, with approximately half of the newly formed urea excreted in the urine and half recycled to the gut. The high correlation coefficients indicate that warm- and cool-season grasses exhibit similar responses in urea production and urinary excretion in steers.

Earlier compilations of data (Sniffen et al., 1992) indicated that a large proportion of N in most forages is NPN, and that this proportion decreases as plants mature. Our forage and those of Archibeque et al. (2001) had from 21 to 31% of total N present as NPN. If we assume that of all the soluble N most of the B_2 fraction is degraded, then between 48 and 72% of the N fed to steers in these two experiments would enter the ruminal ammonia pool. There was a strong relationship between intake of the A fraction and urea production (Figure 4); the slope indicates that each unit increase in A fraction intake is correlated with a 1.7 unit increase in urea N production, ostensibly due to release of ammonia in the rumen and its subsequent absorption and direct incorporation into urea in the liver. There was a positive linear relationship between urea production and B_1 fraction intake ($R^2 = 0.82$, $P < 0.10$, data not shown), and differences ($P < 0.10$) among forages after adjustment for B_1 fraction intake, but quantitatively the B_1 fraction is a minor component in these forages (Table 2; Archibeque et al., 2001). There were forage × N component interactions for both B_2 ($P < 0.07$) and B_3 ($P < 0.01$) fraction intake (Figures 5 and 6). Urea production in response to increased B_2 fraction intake was greater for the warm-season grasses than for tall fescue (Figure 5), and urea production decreased in response to increased B_3 fraction intake from tall fescue but increased with B_3 intake from the warm-season grasses (Figure 6).

In addition to differences among forages in composition of B_2 and B_3 protein fractions, other factor(s) may interact with grass species to alter urea production, excretion, and recycling. These factors likely reside in differences among carbohydrate and lignin fractions of the grasses. The warm-season grasses were 5 to 10% higher in NDF, ADF, and lignin than the cool-season grass.

Implications

Supplementing growing beef steers with ruminally protected methionine can improve the efficiency of N retention. Methionine supplementation improves N retention by means independent of urea production. The response to methionine supplementation may be limited by energy supply. This may be of importance to

producers who must operate within established limits of the amount of N that may remain onsite by increasing the amount of N retained within the steers and therefore removed from the site of production. These data may be applicable to regions where pasture intake is limited, as with these steers, and producers compensate by providing a higher-quality, higher-N-concentration forage. Therefore, steps such as methionine supplementation may be taken to decrease the loss of N in the waste by limit-fed steers.

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